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control proteins. The polypeptides are useful in methods for targeting non-vacuolar harmful proteins to plant vacuoles. Chimeric polypeptides of the invention containing pest control proteins are useful for conferring pest resistance on plants and in the production of compositions useful as pesticides.

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### REMARKS

#### *The Invention*

The present invention is directed to nucleic acids encoding polypeptides comprising vacuole targeting sequence and a plant-noxious pest control protein linked in operable combination to said targeting polypeptide. The plant-noxious pest control sequence is a biotin binding sequence or a functionally equivalent variant or a fragment thereof. In some embodiments, the vacuole targeting sequence is a potato proteinase inhibitor signal sequence.

#### *Status of the Claims*

After entry of this amendment, claims 16-23, 31, and 53-60 are pending. New claims 55-64 have been added. Claims 16-21, 23, 31, and 53-54 are objected to for informalities. Claim 54 stands rejected under 35 U.S.C. §101 as allegedly lacking utility. Claims 16-23, 31, and 53-54 stand rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement; claims 16-23, 31, and 53-54 stand rejected under 35 U.S.C. §112, first paragraph as allegedly lacking adequate written description; claims 16-23, 31, and 53-54 stand rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite; claims 16-23, 31, and 53-54 stand rejected under 35 U.S.C. §103 as allegedly obvious. These rejections are addressed below.

Claims 16-21, 23, 31, and 53-54 have been amended to address alleged informalities and for clarity. Claim 16 has been amended to be in independent form and to recite "wherein the plant-noxious pest control sequence is a biotin binding sequence." Support for this amendment is found in the specification at, *e.g.*, page 11, line 37 and page 13, lines 31-32. Claim 54 has been amended to recite "wherein said seed comprises said nucleic acid molecule." Support for this amendment, is found in the specification at,

*e.g.*, page 23, lines 34-37. New claims 55-64 have been added. Support for these claims is found at, *e.g.*, claim 16, and in the specification at page 11, line 25 to page 12, line 1 page 13, lines 31-32, and page 14, lines 4-19. Thus, no new matter is added by these amendments.

A version of the claims with markings to show changes to the claims are provided in Appendix A. All of the pending claims are provided in Appendix B for the Examiner's convenience.

**Objection to the Title**

The title is objected to as not descriptive of the presently claimed invention. The title has been amended to reflect that the presently pending claims are directed to nucleic acids. Accordingly, Applicants respectfully request that the Examiner withdraw the objections to the title.

**Objection to the Abstract**

The abstract is objected to as not descriptive of the presently claimed invention. The abstract has been amended to reflect that the presently pending claims are directed to nucleic acids. Accordingly, Applicants respectfully request that the Examiner withdraw the objections to the abstract.

**Objection to the Drawings**

The drawing are objected to as indicated on PTO form 948. A set of new drawings accompanies this response. Accordingly, Applicants respectfully request that the Examiner withdraw the objections to the drawings.

**Objection to the Claims**

Claims 16-21, 23, 31, and 53-54 are objected to for alleged informalities. Specifically, claims 16 and 21 allegedly have an improper article before "polypeptide." Claim 16 has been amended to be in independent form and claim 21 has been amended to recite "the polypeptide." Claims 17 and 20 allegedly begin with an improper article. Claims 17 and 20 have been amended to begin with "The." Claims 18, 23, and 53

allegedly have an improper article before “DNA”. Claims 18, 23, and 53 have been amended to recite “the DNA.” Claim 19 allegedly has an improper article before “vector.” Claim 19 has been amended to recite “the vector.” Claim 31 allegedly has an improper article before “chimeric.” Claim 31 has been amended to recite “the chimeric.” Claim 54 allegedly has an improper article before “plant.” Claim 54 has been amended to recite “the plant.”

Accordingly, Applicants respectfully request that the Examiner withdraw the objections to the claims.

**Rejections Under 35 U.S.C. §101**

Claim 54 stands rejected under 35 U.S.C. § 101 as allegedly lacking utility. Claim 54 has been amended in accordance with the Examiner’s suggestion to recite “wherein said seed comprises said nucleic acid molecule.” Accordingly, Applicants respectfully request withdrawal of this rejection.

**Rejections Under 35 U.S.C. §112, first paragraph**

1. Written Description

Claims 16-23, 31, and 53-54 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking adequate written description. In making the rejection, the Examiner acknowledges that there is adequate written description for, *inter alia*, vectors encoding the potato proteinase inhibitor I signal peptide operably linked to avidin mature peptide or potato proteinase inhibitor II signal peptide operably linked to streptavidin, but alleges that the specification contains inadequate written description for nucleic acids that encode a chimeric protein comprising any vacuole targeting sequence linked to any plant-noxious pest control protein.

As set forth in MPEP § 2173.02, “[d]efiniteness of claim language, must be analyzed in light of (A) content of the application; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.”

Applicants respectfully assert that the specification adequately defines the terms or the terms are adequately understood to one of skill in the art, such that the claims are not indefinite under 35 U.S.C. §112, second paragraph. For example, the specification provides examples of multiple vacuole targeting sequences (*i.e.*, sequences targeting barley lectin, sweet potato sporamin, tobacco chitinase, bean phytohemagglutinin, 2S albumin, and aleurain to plant vacuoles) useful in the present invention (*see, e.g.*, page 11, lines 25-35). Thus, one of skill in the art reading the specification would readily understand what is meant by the term vacuole targeting sequence. In addition, solely to expedite prosecution, claim 16 been amended to recite “wherein the plant-noxious pest control sequence is a biotin binding sequence.” Accordingly, Applicants respectfully request withdrawal of this rejection.

## 2. Enablement

Claims 16-23, 31, and 53-54 stand rejected 35 U.S.C. §112, first paragraph as allegedly lacking enablement. In making the rejection, the Examiner acknowledges that the claims are enabled for, *inter alia*, vectors encoding the potato proteinase inhibitor I signal peptide operably linked to avidin mature peptide or potato proteinase inhibitor II signal peptide operably linked to streptavidin, but alleges that the specification does not enable nucleic acids that encode a chimeric protein comprising any vacuole targeting sequence linked to any plant-noxious pest control protein..

A particular claim is enabled by the disclosure in an application if the disclosure, at the time of filing, contains sufficient information so as to enable one of skill in the art to make and use the claimed invention without *undue* experimentation. *See, e.g., In re Wands*, 8 USPQ2d, 1400 (Fed. Cir. 1988), or MPEP §2164.01. It is important to note that the possibility that some experimentation, even if such experimentation is complex or extensive, may be required for the practice of the invention does not necessarily mean that the invention is not enabled:

Applicants respectfully assert that the claims are fully enabled by the specification as originally filed. In particular, the specification provides teachings

regarding (1) multiple vacuole targeting sequences (*i.e.*, sequences targeting barley lectin, sweet potato sporamin, tobacco chitinase, bean phytohemagglutinin, 2S albumin, and aleurain to plant vacuoles) (*see, e.g.*, page 11, lines 25-35); (2) vectors and host cells into which the nucleic acids can be inserted and expressed (*see, e.g.*, page 16, line 29 to page 19, line 1); (3) methods for transfecting cells with the vectors (*see, e.g.*, page 19, lines 3-22); (4) plants suitable for transformation with the vectors (*see, e.g.*, page 20, line 34 to page 21, line 33)); and plants transfected with the vectors (*see, e.g.*, page 20, lines 21-32 and page 21, line 35 to page 22, line 10). Therefore, a skilled artisan, using the teachings of the specification either alone or together with what is known to those of skill in the art, would be able to practice the invention as claimed, without undue experimentation. In addition, solely to expedite prosecution, claim 16 been amended to recite "wherein the plant-noxious pest control sequence is a biotin binding sequence." Accordingly, Applicants respectfully request withdrawal of this rejection.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

**Rejections Under 35 U.S.C. §112, second paragraph**

Claims 16-23, 31, and 53-54 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants respectfully traverse this rejection.

As set forth in MPEP § 2173.02, "[d]efiniteness of claim language, must be analyzed in light of (A) content of the application; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made."

In the instant case, the specification adequately defines the terms or the terms are adequately understood to one of skill in the art, such that the claims are not indefinite under 35 U.S.C. §112, second paragraph. Several bases of indefiniteness were raised, and they will be discussed in turn.

1. Claims 16 and 31

Claim 16 and 31 have been rejected as allegedly indefinite as indefinite for dependency on a nonelected claim. Claim 16 has been amended so that it is in independent form and claim 31 has been amended to depend on claim 16. Accordingly, Applicants respectfully request withdrawal of this rejection.

2. Claims 16 and 21

Claims 16 and 21 have been rejected as allegedly indefinite for lacking antecedent basis for "polypeptide according to claim 1." Claim 16 has been amended so that it is in independent form and claim 21 has been amended to depend on claim 16. Accordingly, Applicants respectfully request withdrawal of this rejection.

3. Claim 17

Claim 17 has been rejected as allegedly indefinite for lacking antecedent basis for "said nucleic acid." Claim 17 has been amended to ensure correct antecedent basis. Accordingly, Applicants respectfully request withdrawal of this rejection.

4. Claims 18, 22-23, and 52

Claims 18, 22-23, and 52 have been rejected as allegedly indefinite for lacking antecedent basis for the recitation "DNA molecule according to claim 17" as claim 17 is drawn to a DNA molecule. Applicants respectfully assert that claim 17 is drawn to nucleic acid molecules that are DNA molecules. Thus, there is adequate antecedent basis for the recitation "DNA molecule according to claim 17." Accordingly, Applicants respectfully request withdrawal of this rejection.

5. Claim 53

Claim 53 has been rejected as allegedly indefinite for the recitation "plant-noxious protein." Claim 53 ultimately depends on claim 16 which has been amended to

recite “wherein the plant-noxious pest control protein is a biotin binding protein.” Therefore, Applicants respectfully assert that one of skill in the art could readily determine the meaning of the recitation “plant-noxious protein.” Accordingly, Applicants respectfully request withdrawal of this rejection.

**Rejections Under 35 U.S.C. § 103**

Claims 16-23, 31, and 53-54 are rejected under 35 U.S.C. § 103 as allegedly unpatentable over Raikel *et al.* (U.S. Patent No. 5,360,726) in view of Czapla *et al.* (WO 94/00992). Claims 16-23, 31, and 53-54 are also rejected under 35 U.S.C. § 103 as allegedly unpatentable over Boller *et al.* (U.S. Patent No. 6,054,637) in view of Hilder *et al.* (*Nature* 330:160-163 (1987)). Each of these rejections is addressed in turn below, in the order raised by the Examiner.

As set forth in M.P.E.P. § 2143, “[t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).”

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. As explained herein below in connection with each of the § 103(a) obviousness rejections, Applicants assert that a *prima facie* case of obviousness has not been established for at least the following reason: the cited art references do not teach or suggest all the claim limitations.

1. Rejection of claims 16-23, 31, and 53-54 under 35 U.S.C. § 103 over Raikel *et al.* in view of Czapla *et al.*

Claims 16-23, 31, and 53-54 have been under 35 U.S.C. § 103 over Raikel *et al.* in view of Czapla *et al.* Specifically, Raikel *et al.* is cited as teaching a methods of targeting lectin or chitinase to plant cell vacuoles and Czapla *et al.* is cited as teaching that plants transformed with nucleic acids encoding avidin or streptavidin are resistant to insect larvae. In making the rejection, the Examiner acknowledges that Raikel *et al.* does not disclose nucleic acids encoding a chimeric protein comprising a lectin vacuole targeting sequence operably linked to avidin or streptavidin.

As explained above, the present invention is directed to a nucleic acid encoding a polypeptide comprising a vacuole targeting sequence and a plant noxious pest control sequence, *i.e.*, a biotin binding sequence or a fragment thereof. In some embodiments, the vacuole targeting sequence is a potato proteinase inhibitor signal sequence.

The combination of Raikel *et al.* and Czapla *et al.* does not teach or suggest all of the elements of the presently claimed invention. Raikel *et al.* discloses amino acid sequences necessary for correct sorting of lectin and chitinase to vacuole and using those sequences to target lectins and chitinases to plant vacuoles. As disclosed in Raikel *et al.*, lectins and chitinases are vacuolar proteins, *i.e.*, proteins that are normally targeted to plant vacuoles. In contrast to the present invention, there is no suggestion or mention in Raikel *et al.* of targeting proteins other than lectins or chitinases to plant vacuoles. Furthermore, there is no disclosure in Raikel *et al.* of targeting **any** proteins to plant vacuoles that are not vacuolar proteins. In particular, Raikel *et al.* does not disclose targeting non-vacuolar proteins (*i.e.*, proteins that are not normally targeted to plant vacuoles such as biotin binding proteins) to plant vacuoles. Czapla *et al.* does not remedy the defect in Raikel *et al.*. Czapla *et al.* discloses transient expression of avidin in maize suspension culture (*see, e.g.*, page 18, lines 16-18). There is no suggestion or mention in Czapla *et al.* of targeting avidin to plant vacuoles. The word “vacuole” does not even appear in Czapla *et al.*



Therefore, the combination of Raikel *et al.* and Czapla *et al.* do not teach or suggest a nucleic acid encoding a polypeptide comprising a vacuole targeting sequence and a plant noxious pest control sequence as disclosed and claimed in the present invention. Absent such a teaching or suggestion, the nucleic acids of the present invention are non-obvious and, thus, patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

2. Rejection of claims 16-23, 31, and 53-54 under 35 U.S.C. § 103 over Boller *et al.* in view of Hilder *et al.*

Claims 16-23, 31, and 53-54 have been rejected under 35 U.S.C. § 103 over Boller *et al.* in view of Hilder *et al.* Specifically, Boller *et al.* is cited as teaching nucleic acids encoding vacuolar signal peptides and using vacuolar signal peptides like cowpea trypsin inhibitors in plant vacuoles. Hilder *et al.* is cited as teaching constructs encoding cowpea trypsin inhibitors and plants transformed with those constructs.

As explained above, the present invention is directed to a nucleic acid encoding a polypeptide comprising a vacuole targeting sequence and a plant noxious pest control sequence, *i.e.*, a biotin binding sequence or a fragment thereof. In some embodiments, the vacuole targeting sequence is a potato proteinase inhibitor signal sequence.

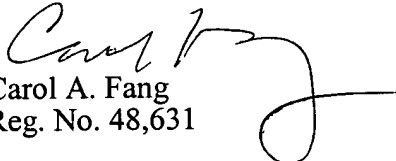
The combination of Boller *et al.* and Hilder *et al.* does not teach or suggest all of the elements of the presently claimed invention. Boller *et al.* discloses chitinase sequences for correct sorting of lectin and chitinase to vacuole and the use of those sequences to target lectins to plant vacuoles. **None** of the sequences disclosed in Boller *et al.* are used to target biotin binding proteins to vacuoles. Hilder *et al.* does not remedy the defect in Boller *et al.*. Hilder *et al.* discloses transfection of tobacco plants with nucleic acids encoding cowpea trypsin inhibitors. There is no suggestion or mention in Hilder *et al.* of targeting any protein to plant vacuoles. Likewise, there is no suggestion or mention in Hilder *et al.* of biotin binding proteins.

Therefore, the combination of Boller *et al.* and Hilder *et al.* do not teach or suggest a nucleic acid encoding a polypeptide comprising a vacuole targeting sequence and a plant noxious pest control sequence as disclosed and claimed in the present invention. Absent such a teaching or suggestion, the nucleic acids of the present invention are non-obvious and, thus, patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, the Examiner is invited to call the undersigned at 415-576-0200.

Respectfully submitted,

  
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**APPENDIX A**

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE TITLE:**

Please replace the title with the following title:

NUCLEIC ACIDS ENCODING CHIMERIC POLYPEPTIDES  
ALLOWING EXPRESSION OF PLANT-NOXIOUS PROTEINS

**IN THE SPECIFICATION:**

Please delete the paragraph beginning at page 8, line 10.

Please delete the paragraph beginning at page 8, line 14.

Please delete the paragraph beginning at page 8, line 28.

Please delete the paragraph beginning at page 8, line 32.

**IN THE ABSTRACT:**

Please replace the abstract with the following abstract:

This invention relates to nucleic acids encoding chimeric polypeptides comprising vacuole targeting sequences and plant-noxious sequences and especially pest control proteins. The polypeptides are useful in methods for targeting non-vacuolar harmful proteins to plant vacuoles. Chimeric polypeptides of the invention containing pest control proteins are useful for conferring pest resistance on plants and in the production of compositions useful as pesticides. [The methods and compositions form further aspects of the invention.]

**IN THE CLAIMS:**

16. (Twice amended) An isolated nucleic acid molecule encoding a polypeptide [according to claim 1], said nucleic acid molecule comprising

(a) a vacuole targeting sequence; and

(b) a plant-noxious pest control sequence linked in operable combination to said vacuole targeting sequence,

wherein the plant-noxious pest control sequence is a biotin binding sequence or a functionally equivalent variant or a fragment thereof.

17. (Once amended) [A] The nucleic acid molecule according to claim 16, wherein said nucleic acid molecule is a DNA molecule.

18. (Once amended) A vector comprising the [a DNA] nucleic acid molecule according to claim 17.

19. (Once amended) A host cell transformed with the [a] vector according to claim 18.

20. (Once amended) [A] The host cell according to claim 19, wherein said cell is a plant cell.

21. (Once amended) A method for producing a polypeptide according to claim [1] 16, comprising the steps of:

- (a) culturing a host cell which has been transformed or transfected with a vector which expresses the encoded polypeptide; and optionally
- (b) recovering the expressed polypeptide.

22. (Once amended) [A] The method for producing a pest resistant plant, comprising transforming the plant genome to include at least one [DNA] nucleic acid molecule according to claim 17.

23. (Once amended) A transgenic plant that contains the [a DNA] nucleic acid molecule according to claim 17.

31. (Once amended) A transgenic plant expressing pesticidally effective concentrations of the [a] chimeric polypeptide according to claim [1] 16.

53. (Once amended) A method for producing a plant-noxious protein, the method comprising extracting the protein from a plant incorporating in its genome the [a DNA] nucleic acid molecule according to claim 17.

54. (Once amended) Seed that is the product of the [a] plant according to claim 23, wherein said seed comprises said nucleic acid molecule.

55. (New) The nucleic acid of claim 16, wherein the vacuole targeting sequence is a potato proteinase inhibitor signal sequence.

56. (New) The nucleic acid of claim 16, wherein the biotin binding sequence is a streptavidin sequence.

57. (New) The nucleic acid of claim 56, wherein the streptavidin sequence is selected from a CORE streptavidin sequence, a synthetic CORE streptavidin sequence, and SYN SAV.

58. (New) The nucleic acid of claim 56, wherein the streptavidin sequence comprises the sequence set forth in SEQ ID NO:10.

59. (New) The nucleic acid of claim 16, wherein the biotin binding sequence is an avidin sequence.

60. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor I signal sequence.

61. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor II signal sequence.

62. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor I signal sequence and the biotin binding sequence is an avidin sequence.

63. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor II signal sequence and the biotin binding sequence is a streptavidin sequence.

64. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is an N-terminal targeting sequence.

**APPENDIX B**  
**PENDING CLAIMS**

16. (Twice amended) An isolated nucleic acid molecule encoding a polypeptide, said nucleic acid molecule comprising

- (a) a vacuole targeting sequence; and
- (b) a plant-noxious pest control sequence linked in operable combination to said vacuole targeting sequence,

wherein the plant-noxious pest control sequence is a biotin binding sequence or a functionally equivalent variant or a fragment thereof.

17. (Once amended) The nucleic acid molecule according to claim 16, wherein said nucleic acid molecule is a DNA molecule.

18. (Once amended) A vector comprising the nucleic acid molecule according to claim 17.

19. (Once amended) A host cell transformed with the vector according to claim 18.

20. (Once amended) The host cell according to claim 19, wherein said cell is a plant cell.

21. (Once amended) A method for producing the [a] polypeptide according to claim 16, comprising the steps of:

- (a) culturing a host cell which has been transformed or transfected with a vector which expresses the encoded polypeptide; and optionally
- (b) recovering the expressed polypeptide.

22. (Once amended) The method for producing a pest resistant plant, comprising transforming the plant genome to include at least one nucleic acid molecule according to claim 17.

23. (Once amended) A transgenic plant that contains the nucleic acid molecule according to claim 17.

24. A transgenic plant according to claim 23, further comprising at least one additional DNA molecule encoding a protein or peptide.

31. (Once amended) A transgenic plant expressing pesticidally effective concentrations of the chimeric polypeptide according to claim 16.

53. (Once amended) A method for producing a plant-noxious protein, the method comprising extracting the protein from a plant incorporating in its genome the nucleic acid molecule according to claim 17.

54. (Once amended) Seed that is the product of the plant according to claim 23, wherein said seed comprises said nucleic acid molecule.

55. (New) The nucleic acid of claim 16, wherein the vacuole targeting sequence is a potato proteinase inhibitor signal sequence.

56. (New) The nucleic acid of claim 16, wherein the biotin binding sequence is a streptavidin sequence.

57. (New) The nucleic acid of claim 56, wherein the streptavidin sequence is selected from a CORE streptavidin sequence, a synthetic CORE streptavidin sequence, and SYNSAV.

58. (New) The nucleic acid of claim 56, wherein the streptavidin sequence comprises the sequence set forth in SEQ ID NO:10.

59. (New) The nucleic acid of claim 16, wherein the biotin binding sequence is an avidin sequence.



60. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor I signal sequence.

61. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor II signal sequence.

62. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor I signal sequence and the biotin binding sequence is an avidin sequence.

63. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is a potato proteinase inhibitor II signal sequence and the biotin binding sequence is a streptavidin sequence.

64. (New) The nucleic acid of claim 55, wherein the vacuole targeting sequence is an N-terminal targeting sequence.